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(54) Low binding affinity Interleukin-12 beta receptors

(57) The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

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Description

This invention relates generally to Interleukin-12 receptors, especially to human Interleukin-12 receptors.

Interleukin-12 (IL-12), formerly known as cytotoxic lymphocyte maturation factor or natural killer cell stimulatory factor, is a 75-kDa heterodimeric cytokine composed of disulfide-bonded 40-kDa (p40) and 35-kDa (p35) subunits that has multiple biological activities including stimulation of the proliferation of activated T and NK cells (Gately, M. K., et al., 1991, *J. Immunol.*, 147:874) (Kobayashi, M., et al., 1989, *J. Exp. Med.*, 170:827), enhancement of the lytic activity of NK/LAK cells (Kobayashi, M., et al., 1989, *J. Exp. Med.*, 170:827; Stern, A.S., et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:6808), enhancement of cytolytic T-cell responses (Gately, M.K., et al., 1992, *Cell. Immunology*, 143:127), induction of interferon gamma by resting and activated T- and NK-cells (Kobayashi, M. et al., 1989, *J. Exp. Med.*, 170:827; Chan, S. H., et al., 1991, *J. Exp. Med.*, 173:869), and promotion of T_H1-type helper cell responses (Manetti, R., et al., 1993, *J. Exp. Med.*, 177:1199; Hsieh, C.-S., et al., 1993, *Science* 260:547).

The biological activity of IL-12 is mediated by the binding of the IL-12 molecules to cell surface, or plasma membrane, receptors on activated T-and NK cells; however, the contributions of the individual subunits, p35 and p40, to receptor binding and signal transduction remain unknown. Studies with labeled IL-12 have shown that this binding occurs in a specific and saturable manner. IL-12 delivers a signal to target cells through a receptor that was initially characterised on phytohaemagglutinin (PHA)-activated CD4+ and CD8+ T-cells and on IL-2 activated CD56+ NK-cells (Chizzonite, R., et al., 1992, *J. Immunol.*, 148:3117; Desai, B., et al., 1992, *J. Immunol.*, 148:3125).

A survey of over 20 human cell lines belonging to the T-, B-, NK- and myelomonocytic lineages only identified a single CD4+, IL-2 dependent human T-cell line (Kit 225/K6) that constitutively expresses the IL-12 receptor and responds to IL-12 (Desai, B., et al., 1992, *J. Immunol.*, 148:3125; Desai, B., et al., 1993, *J. Immunol.* 150:207A). Freshly prepared PHA-activated peripheral blood mononuclear cells (PBMC) and the Kit 225/K6 cell line thus represent two convenient cell sources to study the biochemistry of the functional IL-12 receptor; there may be others.

Equilibrium binding experiments with ¹²⁵I-labeled IL-12 identified three sites with binding affinities for human IL-12 of 5-20 pM, 50-200 pM, and 2-6 nM on IL-12 responsive T-cells (Chizzonite, R., et al., 1994, *Cytokine* 6(5):A82a).

A cDNA encoding a low affinity IL-12 receptor was previously cloned (Chua, A., et al, 1994, *J. Immunology* 153:128; European Patent Application No. 0,638,644). Based on a previously suggested nomenclature (Stahl and Yancopoulos, 1993, *Cell* 74:587), the initially isolated human IL-12 receptor chain is called the beta1 chain.

The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

Brief description of the drawings:

Figure 1: DNA sequence of human IL-12 receptor beta2 cDNA. (start codon = nucleotide 641; stop codon = nucleotide 3226.)(SEQ ID NO:1).

Figure 2: Amino acid sequence of human IL-12 receptor beta2 protein. (single underlined amino acid residues at the N-terminal sequence = signal peptide; amino acid nos. 623-646 = transmembrane area, marked by double underline; 9 potential N-linked glycosylation sites in the extracellular portion are marked by bold italics and are also underlined; conserved box 1 and 2 motifs in the cytoplasmic domain are shaded [amino acid residues nos. 667-669, 699-704, 786-798])(SEQ ID NO:2).

Figure 3: DNA sequence of human IL-12 receptor beta1 cDNA (start codon = nucleotide 65; stop codon = nucleotide 2050)(SEQ ID NO:3).

Figure 4: Amino acid sequence of human IL-12 receptor beta1 protein. (underlined amino acid residues of N-terminal sequence = signal peptide sequence; amino acid residues nos. 541 to 571 = transmembrane area marked by -----; 6 potential N-linked glycosylation sites in the extracellular portion marked by -----; conserved box 1 and 2 motifs in the cytoplasmic domain are marked by ===== [amino acid residues nos. 577 to 584 and 618 to 629])(SEQ ID NO:4).

Figure 5A: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta1 receptor protein.

Figure 5B: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta2 receptor protein.

Figure 5C: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein.

Figure 6: Analysis of proliferation, in the presence of various concentrations of human IL-12, of Ba/F3 cells stably transfected with cDNA for human IL-12 beta1 receptor protein (-- ♦ --), with cDNA for human IL-12 beta2 receptor protein (-- ○ --), or with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein (-- ● --), by measuring incorporation of tritiated thymidine.

The present invention relates to a low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12. In a preferred embodiment of the present invention the IL-12 beta2 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:2 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:1. In a more preferred embodiment the nucleic acid encoding the IL-12 beta2 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:1. Especially, the invention relates to the human IL-12 beta2 receptor protein having for example the amino acid sequence of SEQ ID NO:2 or allelic forms or variants thereof.

In addition, the invention relates to a complex capable of binding to IL-12 with high affinity, comprising the IL-12 beta2 receptor protein, or a fragment thereof as defined above complexed with human IL-12 beta1 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

In a preferred embodiment the above complex comprises an IL-12 beta1 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:4 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:3. In a more preferred embodiment the nucleic acid encoding the IL-12 beta1 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:3. Especially, the invention relates to the human IL-12 beta1 receptor protein having for example the amino acid sequence of SEQ ID NO:4 or allelic forms or variants thereof.

The present invention also relates to the above proteins or complexes which are soluble.

An aspect of the present invention is a protein or complex encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined above, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region. The invention also includes proteins encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of an IL-12 receptor beta2 protein mentioned above and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a IL-12 receptor beta1 protein and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.

The term "human IL-12 beta2 receptor protein" refers to (1) the protein of SEQ ID NO:2, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:2 and which has the following properties:

- 1) The protein or polypeptide has low binding affinity for human IL-12, and
- 2) The protein or polypeptide, when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12.

The term "human IL-12 beta1 receptor protein" refers to (1) the protein of SEQ ID NO:4, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:4 and which has the following properties:

- 1) The protein or polypeptide binds to has low binding affinity for human IL-12, and
- 2) The protein or polypeptide, when complexed with human beta2 receptor protein forms a complex having high binding affinity for human IL-12.

As used herein, the terms "IL-12 beta2 receptor protein" and "IL-12 beta1 receptor protein" includes proteins mod-

ified deliberately, as for example, by site directed mutagenesis or accidentally through mutations. The terms also includes variants which may be prepared from the functional groups occurring as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein and do not confer toxic properties on compositions containing it. These variants may include, for example, polyethylene glycol side-chains which may mask antigenic sites and extend the residence of the proteins in body fluids. Other variants include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl- or threonyl residues) formed with acyl moieties.

"Substantially homologous", which can refer both to nucleic acid and amino acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which do not result in an adverse functional dissimilarity between the reference and subject sequences. For purposes of the present invention, sequences having greater than 80 %, more preferably greater than 90% homology and still more preferably greater than 95% homology, equivalent biological properties, and equivalent expression characteristics are considered substantially homologous. For purposes of determining homology, truncation of the mature sequence should be disregarded. Sequences having lesser degrees of homology, comparable bioactivity, and equivalent expression characteristics are considered substantial equivalents. Generally, homologous DNA sequences can be identified by cross-hybridisation under high stringency hybridisation conditions.

"A fragment of the IL-12 beta2 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of a IL-12 beta2 receptor protein, and which (a) has low binding affinity for IL-12, and (2) when complexed with a IL-12 beta1 receptor protein, forms a complex having high binding affinity for IL-12.

"A fragment of the IL-12 beta1 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of IL-12 beta1 receptor protein, and which when complexed with a IL-12 beta2 receptor protein, forms a complex having high binding affinity for IL-12.

A "soluble fragment" refers to a fragment of a IL-12 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of the protein and which retains the IL-12 binding activity of the intact IL-12 receptor protein. For example, a soluble fragment of a IL-12 beta2 receptor protein is a fragment of a IL-12 beta2 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of a human IL-12 beta2 receptor protein.

In accordance with the invention, a "complex" comprising IL-12 beta2 receptor protein, or a fragment thereof, complexed with IL-12 beta1 receptor protein, or a fragment thereof, may be expressed on the cell surface of the host cell. When expressed on the cell surface of the host cell, the complex has a high binding affinity for IL-12, whereas the IL-12 beta1 receptor protein and the IL-12 beta2 receptor protein alone each have a low binding affinity for IL-12.

In accordance with this invention, the IL-12 beta2 receptor protein may be expressed on the surface of a host cell.

In accordance with this invention, not only the IL-12 beta2 receptor protein may be obtained, but also fragments of IL-12 beta2 receptor protein which (1) have low binding affinity for IL-12 and (2) which when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity. The fragments of IL-12 beta2 receptor protein may be obtained by conventional means, such as (i) proteolytic degradation of the human IL-12 beta2 receptor protein, (ii) chemical synthesis by methods routine in the art, or (iii) standard recombinant methods.

For purposes of the present invention, a human IL-12 receptor protein which has a high binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 5 to about 100 pM. For purposes of the present invention, a human IL-12 receptor protein which has a low binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 1 to about 10 nM. The binding affinity of a protein for IL-12 can be determined by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5.

Fragments of IL-12 beta2 receptor protein can also be measured for binding affinity for IL-12 by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5. The fragments of IL-12 beta2 receptor protein may be measured for binding affinity for IL-12 either alone or complexed with IL-12 beta1 receptor protein, or a fragment of IL-12 beta1 receptor protein which when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity.

The present invention also relates to nucleic acids, e.g. DNA, cDNA, RNA, mRNA, etc. encoding the above proteins, for example a complex capable of binding to human IL-12 with high affinity, the complex comprising human IL-12 beta2 receptor protein, or a fragment thereof, and human IL-12 beta1 receptor protein, or a fragment thereof. Preferably these nucleic acids encode the human IL-12 beta2 receptor protein such as a nucleic acid having the SEQ ID NO:1 and/or the IL-12 beta1 receptor protein such as a nucleic acid having the SEQ ID NO: 3. The present invention also relates to recombinant vectors comprising an above nucleic acid, to expression vectors, and especially to expression vectors wherein the above nucleic acid is operably linked to control sequences recognised by a host cell. The invention includes eukaryotic and prokaryotic host cells transformed with one or more of the above vectors and especially to host

cells wherein the proteins or complexes are expressed on the surface of the host cells and to host cells wherein these cells proliferate in the presence of IL-12. The above host cells may be transformed with a first vector comprising a nucleic acid encoding the IL-12 receptor beta2 protein as defined above and a second vector comprising a nucleic acid encoding the IL-12 receptor beta1 protein as defined above or with a single vector comprising a nucleic acid encoding an IL-12 receptor beta2 protein and a nucleic acid encoding an IL-12 receptor beta1 protein.

As used herein, "nucleic acid" refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from a nucleic acid isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form a DNA or a cDNA with an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

"Expression vector" is a genetic element capable of replication under its own control, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. It comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters and enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences.

"Clone" is a group of identical DNA molecules derived from one original length of DNA sequence and produced by a bacterium or virus using genetic engineering techniques, often involving plasmids.

In addition, the invention refers to a purified, recombinant protein comprising two different polypeptide chains (a heterodimeric protein) which may be prepared by known methods. The two different polypeptide chains are each encoded by a different chimeric polynucleotide which has two nucleic acid subsequences fused in frame. The first nucleic acid subsequence of the first chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of a IL-12 beta2 receptor protein. The second nucleic acid subsequence of the first chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The first nucleic acid subsequence of the second chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of IL-12 beta1 receptor protein. The second nucleic acid subsequence of the second chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region.

The starting materials for the purified, recombinant proteins of the invention may be obtained by methods known in the art. In particular, on the basis of the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 and of the already known nucleic acid sequences for certain receptors, those partial nucleic acid sequences which code for a soluble fragment of IL-12 beta2 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 using known methods, see Sambrook et al., "Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989). Similarly, on the basis of the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 and of the already known DNA sequences for certain receptors, those partial DNA sequences which code for a soluble fragment of human IL-12 beta1 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 using known methods, see Sambrook et al., "Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989). Sources for isolated DNA sequences coding for constant domains of human immunoglobulins are known in the art and disclosed, for example, by Ellison et al., Nucl. Acid Res. 10, 4071-4079 (1982) for IgG₁ or Huck et al., Nucl. Acid Res. 14, 1779-1789 (1986) for IgG₃.

The isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein may be fused in frame, by known methods [Sambrook et al., "Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of the human Ig heavy chain except the first domain of the constant region.

Similarly, the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein may be fused in frame, by known methods [Sambrook et al., "Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of a human Ig heavy chain except the first domain of the constant region.

The chimeric polynucleotides can then be integrated using known methods [Sambrook et al., "Molecular Cloning",

2nd ed., Cold Spring Harbor Laboratory Press (1989)] into suitable expression vectors for expression in a non-human mammalian cell, such as a CHO cell. In order to make the homodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein is integrated into a suitable expression vector. In order to make the heterodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein are integrated into a single suitable expression vector, or two separate suitable expression vectors.

Preferably, the chimeric polynucleotide(s) is/are co-transfected together with a selectable marker, for example neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthin guanine phosphoribosyl transferase (hgpt) using methods which are known in the art. The DNA sequence stably incorporated in the chromosome can subsequently be amplified. A suitable selection marker for this is, for example, dhfr. Mammalian cells, for example, CHO cells, which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transfection has been performed. In this manner, cell lines which contain a higher number of the desired DNA sequence than the unamplified cells can be obtained.

The baculovirus expression system can also be used for the expression of recombinant proteins in insect cells. Postranslational modifications performed by insect cells are very similar to those occurring in mammalian cells. For the production of a recombinant baculovirus which expresses the desired protein a transfer vector is used. A transfer vector is a plasmid which contains the chimeric polynucleotide(s) under the control of a strong promoter, for example, that of the polyhedron gene, surrounded on both sides by viral sequences. The transfer vector is then transfected into the insect cells together with the DNA sequence of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. When using the baculovirus expression system, DNA sequences encoding the immunoglobulin part have to be in the form of cDNA.

The expressed recombinant protein may be purified, for example, by known methods. For example, protein G affinity chromatography may be used to purify the homodimeric protein of the invention. Column chromatography, or any other method that enables differentiation between homodimeric proteins and heterodimeric proteins, may be used to purify the heterodimeric protein of the invention.

Expression of human IL-12 receptor protein having high binding affinity to human IL-12:

The cDNA of cells where the IL-12 receptor is known to be found is incorporated by conventional methods into a bacterial host to establish a cDNA library. PHA-activated PBMC and cells from the Kit 225/K6 cell line are examples of cell sources for the cDNA. RNA from the cells is extracted, characterised, and transcribed into single stranded cDNA by conventional methods. The single stranded cDNA is converted into double stranded cDNA by conventional methods. The double stranded cDNA is incorporated by conventional techniques into an expression vector, such as pEF-BOS. The plasmid DNA from the expression vector is then incorporated into a bacterial host by conventional methods to form a library of recombinants.

The cDNA library is screened by conventional expression screening methods, as described by Hara and Miyajima, 1992, EMBO, 11:1875, for cDNA's which when expressed with cDNA's for the human IL-12 beta1 receptor protein, give rise to a high affinity human IL-12 receptor. A small number of clones from the library are grown in pools. DNA is extracted by conventional methods from the pools of clones. The DNA extracted from a pool of clones is then transfected by conventional methods, along with a small amount of DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein, into non-human host cells. The non-human host cells are preferably mammalian, such as a COS cell. Labeled recombinant human IL-12 is then added to the non-human host cells previously transfected as described above and the binding signal of the pool is determined. This process is repeated for each pool. The pools showing a positive binding signal for IL-12 may then be subsequently broken down into smaller pools and re-assayed in the above manner until a single clone is selected which shows a positive binding signal.

The plasmid DNA from the selected clone is sequenced on both strands using conventional methods, such as an ABI automated DNA sequencer in conjunction with a thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. Amino acid sequence alignments may be run as described by M. O. Dayhoff et al., Methods Enzymology 91:524 (1983) with the mutation data matrix, a break penalty of 6 and 100 random runs.

The DNA from the selected clone is then co-transfected by conventional methods with DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

Alternatively, by conventional recombinant methods, a plasmid may be engineered which contains transcription units (promoter, cDNA, and polyA regions) for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Plasmid DNA is transfected by conventional methods into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

In accordance with this invention, DNA may be isolated which encodes human IL-12 beta2 receptor protein, or a

fragment thereof, which fragment (1) has low binding affinity for human IL-12 and (2) when complexed with human IL-12 beta1 receptor protein, forms a complex having high binding affinity for human IL-12.

An isolated nucleic acid sequence refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from nucleic acid isolated at least once in substantially pure form, that is, free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences, e.g. DNA, are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used as a source of coding sequences. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

In accordance with this invention, a mammalian cell having the human IL-12 beta2 receptor protein or the complex expressed on its surface and which proliferates in response to human IL-12 is useful for determining IL-12 bioactivity. For example, such cells are useful for determining whether a given compound inhibits biological activity of human IL-12 or is an IL-12 agonist.

In addition, through the ability to express the human IL-12 beta2 receptor protein on a non-human mammalian cell surface, we can also express fragments of the human IL-12 beta2 receptor protein, and can determine whether these fragments, when complexed with the beta1 subunit, or an active fragment thereof, have the same properties and high binding affinity for IL-12 as the intact complex.

Isolated DNA encoding the human IL-12 beta2 receptor protein may be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein. The isolated DNA encoding the human IL-12 beta2 receptor protein may also be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor protein with the beta2 receptor protein [See, for example, Charnow, S. M. et al., Trends in Biotechnology, Vol. 14, 52-60(1996)].

Such purified, recombinant proteins, which bind to human IL-12, are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors, by inhibiting binding of IL-12 to such cells. Pathological conditions caused by excess activity of cells possessing IL-12 receptors include autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein is the fusion of a soluble fragment of human IL-12 beta2 receptor protein and a human Ig heavy chain (such as IgG, IgM or IgE, preferably IgG) having all domains except the first domain of the constant region. This recombinant protein, which is homodimeric, is encoded by a chimeric polynucleotide which has 2 DNA subsequences fused in frame. The first DNA subsequence, at the 5' end of the chimeric polynucleotide, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence, located at the 3' end of the chimeric polynucleotide, is an isolated DNA sequence encoding all domains of a human heavy chain Ig (preferably IgG) except the first domain of the constant region. The desired recombinant protein can be generated by transfection of the chimeric polynucleotide into a non-human mammalian cell, such as a chinese hamster ovary (CHO) cell. The expressed recombinant protein can be purified, for example, by protein G affinity chromatography.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor with the beta2 receptor is encoded by two chimeric polynucleotides which each have two DNA subsequences fused in frame. The first DNA subsequence of the first chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence of the first chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The first DNA subsequence of the second chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta1 receptor protein. The second DNA subsequence of the second chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The desired recombinant protein may be generated by cotransfection of the two chimeric polynucleotides into a non human mammalian cell, such as a CHO cell. The expressed protein can be purified, for example, by any method that enables differentiation of homodimeric proteins from heterodimeric proteins, such as, for example, column chromatography.

In addition, the invention also relates to a process for the preparation of a protein mentioned above comprising the expression of an above mentioned nucleic acid in a suitable host cell.

In addition, monoclonal or polyclonal antibodies directed against the human IL-12 beta2 receptor protein, or fragments thereof, or the complex, may also be produced by known methods [See, for example, Current Protocols in Immunology, ed. by Coligan, J.E. et al., J. Wiley & Sons (1992)] and used to prevent or treat pathological conditions caused by excess activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

Purified, recombinant proteins are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

"Purified", as used to define the purity of a recombinant protein encoded by the combined DNA sequences described above, or protein compositions thereof, means that the protein or protein composition is substantially free of other proteins of natural or endogenous origin and contains less than about 1% by mass of protein contaminants residual of production processes. Such compositions, however, can contain other proteins added as stabilizers, carriers, excipients or co-therapeutics. A protein is purified if it is detectable, for example, as a single protein band in a polyacrylamide gel by silver staining.

Purified recombinant proteins as described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered in clinical treatment of autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis.

The purified recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be used in combination with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF (tumor necrosis factor) receptor, the IL-1 antagonist, and the like to treat or prevent the above disorders or conditions.

In addition, the invention relates to pharmaceutical compositions comprising a protein or an antibody mentioned above and a pharmaceutically acceptable carrier. The pharmaceutical compositions may comprise a therapeutically effective amount of one or more cytokine antagonists.

Further, the invention relates to the use of a protein or an antibody mentioned above for the preparation of a medicament. These compounds are especially useful for the treatment of autoimmune dysfunction.

The dose ranges for the administration of the purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

The dose ranges for the administration of the IL-12 receptor proteins and fragments thereof may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The expected dose range is about 1 ng/kg/day to about 10 mg/kg/day. The IL-12 receptor proteins and fragments thereof can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcohol/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishes, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

Assays for determining whether a given compound blocks IL-12 activity:

An aspect of the invention is the use of either the human IL-12 beta2 receptor protein or the complex of this invention as a screening agent for pharmaceuticals. In accordance with this invention, we can determine whether a given compound blocks human IL-12 activity or acts as an agonist of IL-12.

A biological activity of human IL-12 is the stimulation of the proliferation of activated T- and NK-cells. Proliferation of activated T-cells causes alloantigen-induced immune responses, such as allograft rejection (such as skin, kidney, and heart transplants) and graft-versus-host reaction in patients who have received bone marrow transplants. This biological activity of human IL-12 is mediated by the binding of the human IL-12 molecules to cell surface receptors on the

activated T-cells.

A compound that blocks human IL-12 activity would, therefore, inhibit the proliferation of activated T-cells and would be useful to treat or prevent alloantigen induced immune responses.

In order to determine if a compound blocks human IL-12 activity, first, a plurality of cells having expressed on their surface either the human IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 and the given compound. Third, it is determined whether the presence of the given compound inhibits proliferation of the cells.

In order to determine if a compound is an agonist of human IL-12, first, a plurality of cells having expressed on their surface either the IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, and which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 or the given compound. Third, it is determined whether the presence of the given compound stimulates proliferation of the cells.

Examples of cells capable of expressing on their surface the complex, which cells proliferate in the presence of human IL-12 include, without limitation, PHA-activated PBMC, Kit 225/K6 cells, and Ba/F3 cells transfected with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Examples of cells capable of expressing on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, which cells proliferate in the presence of human IL-12 include, without limitation, Ba/F3 cells transfected with cDNA for human IL-12 beta2 receptor protein.

In order to determine whether the presence of the given compound inhibits proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells, having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention, are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added either before or simultaneously with human IL-12 to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with human IL-12 and the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly less than the cells of the standard wells, the compound blocks IL-12 activity.

In order to determine whether the presence of the given compound stimulates proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly more than cells that were not exposed to human IL-12, the compound is an agonist of human IL-12.

Accordingly, the present invention relates to a method for screening of compounds useful for inhibition of IL-12 activity or compounds useful as agonists of IL-12 activity, comprising contacting a compound suspected of inhibiting IL-12 activity or of being an agonist of IL-12 activity, to a protein mentioned above, followed by detection of the biological effect.

The following examples are offered by way of illustration, not by limitation.

EXAMPLES**MATERIALS AND METHODS:****1. Proteins, Plasmids and Strains**

Recombinant human IL-12 (U. Gubler et al., 1991, Proc. Natl. Acad. Sci. USA., 88:4143) was obtained as described therein.

Recombinant human IL-2 (H.W. Lahm et al., 1985, J. Chromatog. 326:357) was obtained as described therein.

The plasmid pEF-BOS is based on a pUC 119 backbone and contains the elongation factor 1 alpha promoter to drive expression of genes inserted at the BstXI site (S. Mizushima and S. Nagata, Nucl. Acids Res., 1990, 18:5322).

The human IL-12 receptor beta1 cDNA in the plasmid pEF-BOS was obtained as described in A. Chua et al., 1994, J. Immunology 153:128 and in European Patent Application Publication No. 0638644.

Electrocompetent *E. coli* DH-10B (S. Grant et al., 1990, Proc. Natl. Acad. Sci USA 87:4645) was obtained from Bethesda Research Laboratory (Bethesda, Maryland).

2. Labeling of Human IL-12 with ^{125}I

Recombinant human IL-12 was labeled with ^{125}I as follows. Iodogen was dissolved in chloroform. 0.05 mg aliquots of Iodogen were dried in 12 x 150 mm borosilicate glass tubes. For radiolabeling, 1.0 mCi $\text{Na}[^{125}\text{I}]$ was added to the iodogen-coated borosilicate glass tube, which also contained 0.05 ml of Tris-iodination buffer (25 mM Tris-HCL pH 7.5, 0.4 M NaCl and 1 mM EDTA) to form a ^{125}I solution. The ^{125}I solution was activated by incubating for 6 minutes at room temperature. The activated ^{125}I solution was transferred to a tube containing 0.05 to 0.1 ml recombinant human IL-12 (31.5 mg) in Tris-iodination buffer. The resulting mixture of the activated ^{125}I solution and the recombinant human IL-12 was incubated for 6 minutes at room temperature. At the end of the incubation, 0.05 ml of Iodogen stop buffer (10 mg/ml tyrosine, 10% glycerol in Dulbecco's phosphate buffered saline (PBS), pH 7.40) was added and reacted for 3 minutes. The resulting mixture was then diluted with 1.0 ml Tris-iodination buffer containing 0.25% bovine serum albumin (BSA), and applied to a Bio-Gel P10DG desalting column for chromatography. The column was eluted with Tris-iodination buffer containing 0.25% BSA. 1 ml fractions containing the eluted peak amounts of labeled recombinant human IL-12 were combined. The combined fractions were diluted to 1×10^8 cpm/ml with 1% BSA in Tris-iodination buffer. Incorporation of ^{125}I into recombinant human IL-12 was monitored by precipitation with trichloroacetic acid (TCA). The TCA precipitable radioactivity (10% TCA final concentration) was typically in excess of 95% of the total radioactivity. The radiospecific activity of the labeled recombinant human IL-12 was typically 1000 to 2000 cpm/fmole.

Example 1**Preparation of Human PHA-activated Lymphoblasts**

Human peripheral blood mononuclear cells (PBMC) were isolated from blood collected from healthy donors as described in Gately et al., J. Natl. Cancer Inst. 69, 1245 (1982). The blood was collected into heparinized syringes, diluted with an equal volume of Hank's balanced salt solution and layered over lymphocyte separation medium (LSM[®] obtained from Organon Teknika Corporation, Durham, North Carolina) in tubes. The tubes were spun at 2000 rpm for 20 minutes at room temperature. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected. Collected PBMC were pelleted at 1500 rpm for 10 minutes through a 15 ml cushion of 20% sucrose in Hank's balanced salt solution. Pelleted PBMC were resuspended in tissue culture medium (1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's medium, supplemented with 0.1 mM nonessential amino acids, 60 mg/ml arginine HCl, 10 mM Hepes buffer, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.05 mM 2-mercaptoethanol, and 1 mg/ml dextrose) (TCM) plus 5% human serum and washed twice in TCM.

The PBMC were then activated to form lymphoblasts. In particular, $0.5 - 1 \times 10^6$ cells/ml in TCM plus 5% human serum plus 0.1% (v/v) PHA-P (Difco, Detroit, MI) were cultured for 3 days at 37°C in a 5% CO_2 atmosphere.

After three days, cell cultures were split 1:1 by volume in TCM plus 5% human serum and 50 U/ml recombinant human IL-2 to yield >95% T-cells. These cells were utilized for preparation of a cDNA library.

Example 2**Extraction and Characterization of RNA**

PBMC isolated as in Example 1, activated with PHA for 2-3 days, were harvested and total RNA was extracted using Guanidine Isothiocyanate/Phenol as described by P. Chomczynski and N. Sacchi, Anal. Biochem., 162:156,

1987. PolyA⁺ RNA was isolated from the total RNA by one batch adsorption to oligo dT latex beads as described (K. Kuribayashi et al., Nucl. Acids Res. Symposium Series 19:61, 1988). The mass yield of this purification was about 4% of polyA⁺ RNA.

5 Example 3

cDNA Library

From the above polyA⁺ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS as follows.

3 mg of polyA⁺ RNA were reverse transcribed into single stranded cDNAs using RNaseH minus reverse transcriptase in the presence of α -³²P-dCTP. The resulting single stranded cDNAs were converted into blunt ended double stranded cDNAs as described by U. Gubler and A. Chua, Essential Molecular Biology Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991. BstXI linkers (A. Aruffo and B. Seed, Proc. Natl. Acad. Sci (USA) 84, 8573, 1987) were ligated to the resulting double stranded cDNAs.

cDNA molecules having a size of greater than 800 base pairs (bp) were selected by size exclusion chromatography as follows. A Sephacryl SF 500 column (0.8 x 29 cm) was packed by gravity in 10 mM Tris-HCl pH 7.8 - 1 mM EDTA - 100 mM NaAcetate. The radioactive cDNA with added BstXI linkers was applied to the column and 0.5 ml fractions were collected. The size distribution of radioactive cDNA was determined by performing electrophoresis on a small aliquot of each fraction on a 1% agarose gel, drying the gel, and visualizing the size by exposure of the gel to X-ray film. cDNA molecules larger than 800 bp were size selected in this fashion.

The selected cDNA molecules were pooled and concentrated by ethanol precipitation. The pooled and concentrated selected cDNA molecules were subsequently ligated to the plasmid pEF-BOS as follows. The plasmid had been restricted with BstXI and purified over two consecutive 1% agarose gels. 300 ng of the restricted and purified plasmid DNA were ligated to 30 ng of size selected cDNA in 60 ml of ligation buffer (50 mM Tris-HCl pH 7.8 - 10 mM MgCl₂ - 10 mM DTT - 1 mM rATP - 25 mg/ml BSA) at 15°C overnight.

The following day, the plasmid ligated with the size selected cDNA was extracted with phenol. 6 mg of mussel glycogen were added to the resulting extract, and the nucleic acids were precipitated by ethanol. The resulting precipitate was dissolved in water and the nucleic acids again were precipitated by ethanol, followed by a wash with 80% ethanol. A pellet was formed from the precipitated and washed nucleic acids. The pellet was dissolved in 6 ml of water. 1 ml aliquots of the dissolved pellet were subsequently electroporated into *E. Coli* strain DH-10B. Upon electroporation of 5 parallel aliquots, a library of about 10 million recombinants was generated.

Example 4

Expression Screening for cDNAs Encoding High Affinity IL-12 Receptors

The library was screened according to the general expression screening method described by Hara and Miyajima, 1992, EMBO, 11:1875.

Pools of about 100 *E. coli* clones from the above library were grown and the plasmid DNA was extracted from the pools by conventional methods. 2×10^5 COS cells were plated per 35 mm culture well. COS cells were transfected with a transfection cocktail using the standard DEAE dextran technique described in "Molecular Cloning, a Laboratory Manual", 2nd Ed., J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989 ("Molecular Cloning"). The transfection cocktail contained (1) 1 mg of plasmid DNA extracted from the *E. Coli* clone pools derived from the above library, and (2) 0.1 mg of pEF-BOS plasmid DNA containing the human IL-12 receptor beta1 cDNA.

3 days after transfection, the wells of COS cells were incubated with 10 pM labeled human recombinant IL-12 (specific activity = 1000-2000 cpm/fmole) for 90 minutes at room temperature. The labeled human recombinant IL-12 was removed, and the COS cell monolayer was washed for one hour three times with binding buffer (RPMI 1640, 5% fetal bovine serum (FBS), 25 mM HEPES pH 7) to further select for COS cells expressing high affinity IL-12 receptors only (the binding of the IL-12 ligand to the low affinity sites was further reduced because the low affinity sites have a higher dissociation rate). Subsequently, the cell monolayers were lysed and counted in a gamma counter. After screening 440 pools (representing about 44,000 clones), one pool consistently showed a positive binding signal (300 cpm over 100 cpm background). From this pool, a single clone was subsequently isolated by sib-selection. This single clone (B5-10) contained a cDNA insert of about 3 kb that was completely sequenced.

The cDNA insert of clone B5-10 was incomplete with regard to the protein coding region because it did not contain an in-frame stop codon. The cDNA library of Example 3 was rescreened by conventional DNA hybridization techniques with the cDNA insert from clone B5-10, as described in Molecular Cloning and by Grunstein and Hogness, 1975, Proc. Nat. Acad. Sci. USA., 72:3961. Additional clones were thus isolated and then partially sequenced. The nucleotide sequence of one clone (No. 3) was found to (i) overlap with the 3' end of the nucleotide sequence of clone B5-10, (ii)

extend beyond the nucleotide sequence of clone B5-10, and (iii) contain an in-frame stop codon.

This composite DNA sequence is shown in Figure 1 (SEQ ID NO:1). The deduced amino acid sequence for the encoded receptor protein is shown in Figure 2. Based on the previously suggested nomenclature of Stahl and Yancopoulos, 1993, Cell 74:587, we call this newly isolated human IL-12 receptor chain the beta2 chain.

Example 5

Binding Assays

COS cells ($4-5 \times 10^7$) were transfected by electroporation using a BioRad Gene Pulser (250 mF, 250 volts) with either (1) 25 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein, (2) 25 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein, or (3) a mixture of 12.5 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and 12.5 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein. The electroporated cells were plated in a 600 cm^2 culture plate, harvested after 72 hours by scraping, washed and resuspended in binding buffer.

The cells were assayed to determine affinities of the expressed IL-12 receptors for human IL-12. In particular, equilibrium binding of labeled recombinant human IL-12 to the cells was performed and analyzed as described by R. Chizzonite, et al., 1992, J. Immunol., 148:3117. Electroporated cells (8×10^4) were incubated with increasing concentrations of ^{125}I -labeled recombinant human IL-12 at room temperature for 2 hours. Incubations were carried out in duplicate or triplicate.

Cell bound radioactivity was separated from free labeled ^{125}I -IL-12 by centrifugation of the mixture of electroporated cells and ^{125}I -labeled recombinant human IL-12 through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 (A.H. Thomas) and Silicone Oil AR 200 (Gallard-Schlessinger)) at 4°C for 90 seconds at $10,000 \times g$ to form a cell pellet in a tube. The cell pellet was excised from the tip of the tube in which it was formed, and cell bound radioactivity was determined in a gamma counter.

Receptor binding data were analyzed and the affinities were calculated according to Scatchard using the method described by McPherson, J., 1985, Pharmacol. Methods, 14:213.

Example 6

Production of IL-12 Responsive Cell Line

Wild-type Ba/F3 cells, an IL-3-dependent mouse pro-B cell (Palacios, R. et al., 1985, Cell 41:727) and Ba/F3 cells expressing human IL-12 beta1 receptor protein (Chua, A., et al., 1994, J. Immunology 153:128) were cotransfected with (1) 80 mg of pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and (2) 8 mg of a plasmid expressing a hygromycin resistance gene (Giordano, T.J., et al., 1990, Gene 88:285) by electroporation using a BioRad Gene Pulser (960 mF, 400 volts).

All cells were resuspended at a density of 2×10^5 viable cells/ml in a growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and 10% conditioned medium from the WEHI-3 cell line (ATCC No. TIB 68, American Type Culture Collection, Rockville, Maryland). The WEHI-3 cell line is a source of IL-3. The resuspended cells were then incubated at 37°C under 5% CO_2 for 120 hours.

Cells were selected by their ability to grow in (1) the above growth medium in the presence of 1 mg/ml hygromycin or (2) an IL-12 containing growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and various concentrations (10, 50 or 250 ng/ml) of human IL-12.

Ba/F3 cells expressing human IL-12 beta1 receptor protein transfected with pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein grew in the IL-12 containing growth medium, demonstrating that coexpression of human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Additionally, Ba/F3 cells expressing human IL-12 beta2 receptor protein grow in the IL-12 containing growth medium, demonstrating that expression of human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Example 7

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, or (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin G, 100 mg/ml streptomycin, and 2 mM L-glutamine at

2 x 10⁴ cells/well in Costar 3596 flat-bottom microplates for 24 hours. Various dilutions of human IL-12, as shown in Figure 6, were then added to the microplates and the cells were incubated for 42 hours at 37°C in a humidified atmosphere of 5% CO₂ in air. 50 ml of ³H-thymidine, 10 mCi/ml in culture medium, was then added to each well. The cultures were further incubated for 6 hours at 37°C. Subsequently, the culture contents were harvested onto glass fiber filters by means of a cell harvester. ³H-thymidine incorporation was measured by use of a liquid scintillation counter. All samples were assayed in quadruplicate.

Example 8

Sequence Analysis of IL-12 Receptor cDNA Clones and Encoded IL-12 Receptor Protein

The IL-12 beta2 receptor protein, composed of 862 amino acids and a calculated molecular weight of 97231, had the following features: N-terminal signal peptide, extracellular domain, transmembrane domain and cytoplasmic tail. The classical hydrophobic N-terminal signal peptide is predicted to be 23 amino acids in length. Signal peptide cleavage occurs mostly after the amino acids Ala, Ser, Gly, Cys, Thr, Gln (von Heijne, G., 1986, Nucleic Acids Research, 14:4683). For the IL-12 receptor, the cleavage could thus take place after Ala23 in the sequence shown in Figure 2, leaving a mature protein of 839 amino acids based on cleavage at Ala23. The extracellular domain of the receptor is predicted to encompass the region from the C-terminus of the signal peptide to amino acid No. 622 in the sequence shown in Figure 2. Hydrophobicity analysis shows the area from amino acid No. 623 to 646 to be hydrophobic, as would be expected for a transmembrane anchor region. Charged transfer stop residues can be found at the N- as well as the C-terminus of this predicted transmembrane area. The extracellular domain of the receptor is thus 599 amino acids long and contains 9 predicted N-linked glycosylation sites. The cytoplasmic portion is 215 amino acids long (amino acid residue nos. 647 to 862).

Further analysis of the amino acid sequence shown in Figure 2 shows the human IL-12 beta2 receptor protein is a member of the cytokine receptor superfamily, by virtue of the sequence motifs [Cys132 -- Cys143TW] and [W305SKWS]. Comparing the sequence shown in Figure 2 to all the members of the superfamily by running the ALIGN program shows that the human IL-12 beta2 receptor protein has the highest homology to human gp130. The cytoplasmic region of the IL-12 receptor beta2 chain contains the box 1 and 2 motifs found in other cytokine receptor superfamily members, as well as three tyrosine residues. Phosphorylation of tyrosines is commonly associated with cytokine receptor signalling; the presence of these tyrosine residues underscores the importance of the IL-12 receptor beta2 chain in the formation of a functional IL-12 receptor. The IL-12 receptor beta1 chain does not contain any tyrosine residues in its cytoplasmic tail.

Example 9

Analysis of the Binding Assays

The results of the binding assays are shown in Figure 5.

As shown in Figures 5A and 5B, human IL-12 binds to recombinant IL-12 receptor beta1 or beta2 alone with an apparent affinity of about 2-5 nM. The binding data was described by a single site receptor model, corresponding to the low affinity component of the functional IL-12 receptor found on PHA-activated PBMC (R. Chizzonite et al., 1992, J. Immunol., 148:3117; B. Desai et al., 1992, J. Immunol., 148:3125).

In contrast to these results, as shown in Figure 5C, both high and low affinity IL-12 binding sites were generated upon cotransfection of COS cells with IL-12 receptor beta1 and beta2 plasmids. In this case, the binding data were described by a two receptor site model, with affinities of 50 pM and 5 nM.

Example 10

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

The results of the proliferation assay for the effect of human IL-12 on Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, and (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are shown in Figure 6.

Cells that are transfected with cDNAs for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Additionally, cells that are transfected with cDNAs for human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Consequently, isolated cDNA (clone No. B5-10, SEQ.ID. No:1) coding for a type I transmembrane protein represents a second component of the IL-12 receptor (IL-12R beta2) found on normal human T-cells. The beta1 and beta2

chains each alone bind IL-12 only with low affinity ($K_d = 2-5$ nM). Upon coexpression of beta1 and beta2, two affinity sites are observed, with K_d values of 50 pM and 5 nM.

Ba/F3 cells expressing human IL-12 beta2 receptor protein or coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are responsive to human IL-12.

5 The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: HOFFMANN-LA ROCHE AG
- (B) STREET: Grenzacherstrasse 124
- (C) CITY: Basle
- (D) STATE: BS
- (E) COUNTRY: Switzerland
- (F) POSTAL CODE (ZIP): CH-4002
- (G) TELEPHONE: 061-688 51 08
- (H) TELEFAX: 061-688 13 95
- (I) TELEX: 962292/965542 hlr ch

(ii) TITLE OF INVENTION: RECEPTORS FOR INTERLEUKIN-12

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4040 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 641..3226

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25	TGCAGAGAAC AGAGAAAGGA CATCTGCGAG GAAAGTTCCC TGATGGCTGT CAACAAAGTG	60
	CCACGTCTCT ATGGCTGTGT ACGCTGAGCA CACGATTTTA TCGCGCTAT CATATCTTGG	120
30	TGCATAAACG CACCTCACCT CGGTCAACCC TTGCTCCGTC TTATGAGACA GGCTTTATTA	180
35	TCCGCATTTT ATATGAGGGG AATCTGACGG TGGAGAGAGA ATTATCTTGC TCAAGGCGAC	240
	ACAGCAGAGC CCACAGGTGG CAGAATCCCA CCCGAGCCCG CTTGACCCG CGGGGTGGAA	300
40	ACCACGGGCG CCCGCCGGC TGCGCTTCCA GAGCTGAACT GAGAAGCGAG TCCTCTCCGC	360
45	CCTGCGGCCA CCGCCCAGCC CCGACCCCG CCCC GGCCCG ATCCTCACTC GCCGCCAGCT	420
	CCCCGCGCCC ACCCCGAGT TGGTGGCGCA GAGGCGGGAG GCGGAGGCGG GAGGGCGGGC	480
50	GCTGGCACCG GGAACGCCCC AGCGCCGGCA GAGAGCGCGG AGAGCGCGAC ACGTGCGGCC	540

	CAGAGCACCG GGGCCACCCG GTCCCCGAG GCCCGGGACC GCGCCCGCTG GCAGGCGACA	600
5	CGTGAAGAA TACGGAGTTC TATACCAGAG TTGATTGTTG ATG GCA CAT ACT TTT	655
	Met Ala His Thr Phe	
	1 5	
10	AGA GGA TGC TCA TTG GCA TTT ATG TTT ATA ATC ACG TGG CTG TTG ATT	703
	Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile Thr Trp Leu Leu Ile	
	10 15 20	
15	AAA GCA AAA ATA GAT GCG TGC AAG AGA GGC GAT GTG ACT GTG AAG CCT	751
	Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp Val Thr Val Lys Pro	
20	25 30 35	
	TCC CAT GTA ATT TTA CTT GGA TCC ACT GTC AAT ATT ACA TGC TCT TTG	799
25	Ser His Val Ile Leu Leu Gly Ser Thr Val Asn Ile Thr Cys Ser Leu	
	40 45 50	
	AAG CCC AGA CAA GGC TGC TTT CAC TAT TCC AGA CGT AAC AAG TTA ATC	847
30	Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg Arg Asn Lys Leu Ile	
	55 60 65	
35	CTG TAC AAG TTT GAC AGA AGA ATC AAT TTT CAC CAT GGC CAC TCC CTC	895
	Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His His Gly His Ser Leu	
	70 75 80 85	
40	AAT TCT CAA GTC ACA GGT CTT CCC CTT GGT ACA ACC TTG TTT GTC TGC	943
	Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr Thr Leu Phe Val Cys	
	90 95 100	
45	AAA CTG GCC TGT ATC AAT AGT GAT GAA ATT CAA ATA TGT GGA GCA GAG	991
	Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln Ile Cys Gly Ala Glu	
50	105 110 115	

55

5 ATC TTC GTT GGT GTT GCT CCA GAA CAG CCT CAA AAT TTA TCC TGC ATA 1039
 Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln Asn Leu Ser Cys Ile
 120 125 130

10 CAG AAG GGA GAA CAG GGG ACT GTG GCC TGC ACC TGG GAA AGA GGA CGA 1087
 Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr Trp Glu Arg Gly Arg
 135 140 145

15 GAC ACC CAC TTA TAC ACT GAG TAT ACT CTA CAG CTA AGT GGA CCA AAA 1135
 Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln Leu Ser Gly Pro Lys
 150 155 160 165

20 AAT TTA ACC TGG CAG AAG CAA TGT AAA GAC ATT TAT TGT GAC TAT TTG 1183
 Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile Tyr Cys Asp Tyr Leu
 170 175 180

25 GAC TTT GGA ATC AAC CTC ACC CCT GAA TCA CCT GAA TCC AAT TTC ACA 1231
 Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro Glu Ser Asn Phe Thr
 185 190 195

30 GCC AAG GTT ACT GCT GTC AAT AGT CTT GGA AGC TCC TCT TCA CTT CCA 1279
 Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser Ser Ser Ser Leu Pro
 200 205 210

35 TCC ACA TTC ACA TTC TTG GAC ATA GTG AGG CCT CTT CCT CCG TGG GAC 1327
 Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro Leu Pro Pro Trp Asp
 215 220 225

40 ATT AGA ATC AAA TTT CAA AAG GCT TCC GTG AGC AGA TGT ACC CTT TAT 1375
 Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser Arg Cys Thr Leu Tyr
 230 235 240 245

45 TGG AGA GAT GAG GGA CTG GTA CTG CTT AAT CGA CTC AGA TAT CGG CCC 1423
 Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg Leu Arg Tyr Arg Pro

	250	255	260	
5	AGT AAC AGC AGG CTC TGG AAT ATG GTT AAT GTT ACA AAG GCC AAA GGA			1471
	Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val Thr Lys Ala Lys Gly			
	265	270	275	
10	AGA CAT GAT TTG CTG GAT CTG AAA CCA TTT ACA GAA TAT GAA TTT CAG			1519
	Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr Glu Tyr Glu Phe Gln			
	280	285	290	
15	ATT TCC TCT AAG CTA CAT CTT TAT AAG GGA AGT TGG AGT GAT TGG AGT			1567
	Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser Trp Ser Asp Trp Ser			
20	295	300	305	
25	GAA TCA TTG AGA GCA CAA ACA CCA GAA GAA GAG CCT ACT GGG ATG TTA			1615
	Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu Pro Thr Gly Met Leu			
	310	315	320	325
30	GAT GTC TGG TAC ATG AAA CGG CAC ATT GAC TAC AGT AGA CAA CAG ATT			1663
	Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr Ser Arg Gln Gln Ile			
	330	335	340	
35	TCT CTT TTC TGG AAG AAT CTG AGT GTC TCA GAG GCA AGA GGA AAA ATT			1711
	Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu Ala Arg Gly Lys Ile			
	345	350	355	
40	CTC CAC TAT CAG GTG ACC TTG CAG GAG CTG ACA GGA GGG AAA GCC ATG			1759
	Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr Gly Gly Lys Ala Met			
45	360	365	370	
50	ACA CAG AAC ATC ACA GGA CAC ACC TCC TGG ACC ACA GTC ATT CCT AGA			1807
	Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr Thr Val Ile Pro Arg			
	375	380	385	

55

5 ACC GGA AAT TGG GCT GTG GCT GTG TCT GCA GCA AAT TCA AAA GGC AGT 1855
 Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala Asn Ser Lys Gly Ser
 390 395 400 405

10 TCT CTG CCC ACT CGT ATT AAC ATA ATG AAC CTG TGT GAG GCA GGG TTG 1903
 Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu Cys Glu Ala Gly Leu
 410 415 420

15 CTG GCT CCT CGC CAG GTC TCT GCA AAC TCA GAG GGC ATG GAC AAC ATT 1951
 Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu Gly Met Asp Asn Ile
 425 430 435

20 CTG GTG ACT TGG CAG CCT CCC AGG AAA GAT CCC TCT GCT GTT CAG GAG 1999
 Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro Ser Ala Val Gln Glu
 440 445 450

25 TAC GTG GTG GAA TGG AGA GAG CTC CAT CCA GGG GGT GAC ACA CAG GTC 2047
 Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly Gly Asp Thr Gln Val
 455 460 465

30 CCT CTA AAC TGG CTA CGG AGT CGA CCC TAC AAT GTG TCT GCT CTG ATT 2095
 Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn Val Ser Ala Leu Ile
 470 475 480 485

35 TCA GAG AAC ATA AAA TCC TAC ATC TGT TAT GAA ATC CGT GTG TAT GCA 2143
 Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu Ile Arg Val Tyr Ala
 490 495 500

40 CTC TCA GGG GAT CAA GGA GGA TGC AGC TCC ATC CTG GGT AAC TCT AAG 2191
 Leu Ser Gly Asp Gln Gly Gly Cys Ser Ser Ile Leu Gly Asn Ser Lys
 505 510 515

45 CAC AAA GCA CCA CTG AGT GGC CCC CAC ATT AAT GCC ATC ACA GAG GAA 2239
 His Lys Ala Pro Leu Ser Gly Pro His Ile Asn Ala Ile Thr Glu Glu

	520	525	530	
5	AAG GGG AGC ATT TTA ATT TCA TGG AAC AGC ATT CCA GTC CAG GAG CAA			2287
	Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile Pro Val Gln Glu Gln			
	535	540	545	
10	ATG GGC TGC CTC CTC CAT TAT AGG ATA TAC TGG AAG GAA CGG GAC TCC			2335
	Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp Lys Glu Arg Asp Ser			
	550	555	560	565
15	AAC TCC CAG CCT CAG CTC TGT GAA ATT CCC TAC AGA GTC TCC CAA AAT			2383
	Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr Arg Val Ser Gln Asn			
20		570	575	580
	TCA CAT CCA ATA AAC AGC CTG CAG CCC CGA GTG ACA TAT GTC CTG TGG			2431
25	Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val Thr Tyr Val Leu Trp			
	585	590	595	
30	ATC ACA GCT CTG ACA GCT GCT GGT GAA AGT TCC CAC GGA AAT GAG AGG			2479
	Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser His Gly Asn Glu Arg			
	600	605	610	
35	GAA TTT TGT CTG CAA GGT AAA GCC AAT TGG ATG GCG TTT GTG GCA CCA			2527
	Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met Ala Phe Val Ala Pro			
	615	620	625	
40	AGC ATT TGC ATT GCT ATC ATC ATG GTG GGC ATT TTC TCA ACG CAT TAC			2575
	Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile Phe Ser Thr His Tyr			
	630	635	640	645
45	TTC CAG CAA AAG GTG TTT GTT CTC CTA GCA GCC CTC AGA CCT CAG TGG			2623
	Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala Leu Arg Pro Gln Trp			
50		650	655	660

55

5 TGT AGC AGA GAA ATT CCA GAT CCA GCA AAT AGC ACT TGC GCT AAG AAA 2671
 Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser Thr Cys Ala Lys Lys
 665 670 675

10 TAT CCC ATT GCA GAG GAG AAG ACA CAG CTG CCC TTG GAC AGG CTC CTG 2719
 Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro Leu Asp Arg Leu Leu
 680 685 690

15 ATA GAC TGG CCC ACG CCT GAA GAT CCT GAA CCG CTG GTC ATC AGT GAA 2767
 Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro Leu Val Ile Ser Glu
 695 700 705

20 GTC CTT CAT CAA GTG ACC CCA GTT TTC AGA CAT CCC CCC TGC TCC AAC 2815
 Val Leu His Gln Val Thr Pro Val Phe Arg His Pro Pro Cys Ser Asn
 710 715 720 725

25 TGG CCA CAA AGG GAA AAA GGA ATC CAA GGT CAT CAG GCC TCT GAG AAA 2863
 Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His Gln Ala Ser Glu Lys
 730 735 740

30 GAC ATG ATG CAC AGT GCC TCA AGC CCA CCA CCT CCA AGA GCT CTC CAA 2911
 Asp Met Met His Ser Ala Ser Ser Pro Pro Pro Pro Arg Ala Leu Gln
 745 750 755

35 GCT GAG AGC AGA CAA CTG GTG GAT CTG TAC AAG GTG CTG GAG AGC AGG 2959
 Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys Val Leu Glu Ser Arg
 760 765 770

40 GGC TCC GAC CCA AAG CCA GAA AAC CCA GCC TGT CCC TGG ACG GTG CTC 3007
 Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys Pro Trp Thr Val Leu
 775 780 785

45 CCA GCA GGT GAC CTT CCC ACC CAT GAT GGC TAC TTA CCC TCC AAC ATA 3055
 Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr Leu Pro Ser Asn Ile

56

	790	795	800	805	
5	GAT GAC CTC CCC TCA CAT GAG GCA CCT CTC GCT GAC TCT CTG GAA GAA				3103
	Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala Asp Ser Leu Glu Glu				
		810	815	820	
10	CTG GAG CCT CAG CAC ATC TCC CTT TCT GTT TTC CCC TCA AGT TCT CTT				3151
	Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe Pro Ser Ser Ser Leu				
		825	830	835	
15	CAC CCA CTC ACC TTC TCC TGT GGT GAT AAG CTG ACT CTG GAT CAG TTA				3199
	His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu Thr Leu Asp Gln Leu				
20		840	845	850	
	AAC ATG AGG TGT GAC TCC CTC ATG CTC TGAGTGGTGA GGCTTCAAGC				3246
25	Lys Met Arg Cys Asp Ser Leu Met Leu				
		855	860		
30	CTTAAAGTCA GTGTGCCCTC AACCAGCACA GCCTGCCCCA ATTCCCCCAG CCCCTGCTCC				3306
	AGCAGCTGTC ATCTCTGGGT GCCACCATCG GTCTGGCTGC AGCTAGAGGA CAGGCAAGCC				3366
35	AGCTCTGGGG GAGTCTTAGG AACTGGGAGT TGGTCTTCAC TCAGATGCCT CATCTTGCCT				3426
	TTCCCAGGGC CTTAAAATTA CATCCTTCAC TGTGTGGACC TAGAGACTCC AACTTGAATT				3486
40	CCTAGTAACT TTCTTGGTAT GCTGGCCAGA AAGGGAAATG AGGAGGAGAG TAGAAACCAC				3546
	AGCTCTTAGT AGTAATGGCA TACAGTCTAG AGGACCATTC ATGCAATGAC TATTCTAAA				3606
45	GCACCTGCTA CACAGCAGGC TGTACACAGC AGATCAGTAC TGTTCACAG AACTTCCTGA				3666
50	GATGATGGAA ATGTTCTACC TCTGCACTCA CTGTCCAGTA CATTAGACAC TAGGCACATT				3726
55					

GGCTGTTAAT CACTTGGAAT GTGTTTAGCT TGACTGAGGA ATTAAATTTT GATTGTAAAT 3786

5 TTAAATCGCC ACACATGGCT AGTGGCTACT GTATTGGAGT GCACAGCTCT AGATGGCTCC 3846

TAGATTATTG AGAGCCTCCA AAACAAATCA ACCTAGTTCT ATAGATGAAG ACATAAAAGA 3906

10 CACTGGTAAA CACCAATGTA AAAGGGCCCC CAAGGTGGTC ATGACTGGTC TCATTGTCAG 3966

15 AAGTCTAAGA ATGTACCTTT TTCTGGCCGG GCGTGGTAGC TCATGCCTGT AATCCCAGCA 4026

CTTTGGGAGG CTGA 4040

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 862 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala His Thr Phe Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile

1

5

10

15

Thr Trp Leu Leu Ile Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp

20

25

30

Val Thr Val Lys Pro Ser His Val Ile Leu Leu Gly Ser Thr Val Asn

35

40

45

Ile Thr Cys Ser Leu Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg

	50	55	60
5	Arg Asn Lys Leu Ile Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His		
	65	70	75 80
10	His Gly His Ser Leu Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr		
	85	90	95
15	Thr Leu Phe Val Cys Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln		
	100	105	110
20	Ile Cys Gly Ala Glu Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln		
	115	120	125
25	Asn Leu Ser Cys Ile Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr		
	130	135	140
30	Trp Glu Arg Gly Arg Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln		
	145	150	155 160
35	Leu Ser Gly Pro Lys Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile		
	165	170	175
40	Tyr Cys Asp Tyr Leu Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro		
	180	185	190
45	Glu Ser Asn Phe Thr Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser		
	195	200	205
50	Ser Ser Ser Leu Pro Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro		
	210	215	220
55	Leu Pro Pro Trp Asp Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser		
	225	230	235 240

Arg Cys Thr Leu Tyr Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg

245

250

255

5

Leu Arg Tyr Arg Pro Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val

260

265

270

10

Thr Lys Ala Lys Gly Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr

275

280

285

15

Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser

290

295

300

20

Trp Ser Asp Trp Ser Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu

305

310

315

320

25

Pro Thr Gly Met Leu Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr

325

330

335

30

Ser Arg Gln Gln Ile Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu

340

345

350

35

Ala Arg Gly Lys Ile Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr

355

360

365

40

Gly Gly Lys Ala Met Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr

370

375

380

45

Thr Val Ile Pro Arg Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala

385

390

395

400

50

Asn Ser Lys Gly Ser Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu

405

410

415

55

Cys Glu Ala Gly Leu Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu

420

425

430

Gly Met Asp Asn Ile Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro

435

440

445

Ser Ala Val Gln Glu Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly

450

455

460

Gly Asp Thr Gln Val Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn

465

470

475

480

Val Ser Ala Leu Ile Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu

485

490

495

Ile Arg Val Tyr Ala Leu Ser Gly Asp Gln Gly Gly Cys Ser Ser Ile

500

505

510

Leu Gly Asn Ser Lys His Lys Ala Pro Leu Ser Gly Pro His Ile Asn

515

520

525

Ala Ile Thr Glu Glu Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile

530

535

540

Pro Val Gln Glu Gln Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp

545

550

555

560

Lys Glu Arg Asp Ser Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr

565

570

575

Arg Val Ser Gln Asn Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val

580

585

590

Thr Tyr Val Leu Trp Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser

	595	600	605
5	His Gly Asn Glu Arg Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met		
	610	615	620
10	Ala Phe Val Ala Pro Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile		
	625	630	635 640
15	Phe Ser Thr His Tyr Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala		
	645	650	655
20	Leu Arg Pro Gln Trp Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser		
	660	665	670
25	Thr Cys Ala Lys Lys Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro		
	675	680	685
30	Leu Asp Arg Leu Leu Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro		
	690	695	700
35	Leu Val Ile Ser Glu Val Leu His Gln Val Thr Pro Val Phe Arg His		
	705	710	715 720
40	Pro Pro Cys Ser Asn Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His		
	725	730	735
45	Gln Ala Ser Glu Lys Asp Met Met His Ser Ala Ser Ser Pro Pro Pro		
	740	745	750
50	Pro Arg Ala Leu Gln Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys		
	755	760	765
55	Val Leu Glu Ser Arg Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys		
	770	775	780

Pro Trp Thr Val Leu Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr
785 790 795 800

Leu Pro Ser Asn Ile Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala
805 810 815

Asp Ser Leu Glu Glu Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe
820 825 830

Pro Ser Ser Ser Leu His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu
835 840 845

Thr Leu Asp Gln Leu Lys Met Arg Cys Asp Ser Leu Met Leu
850 855 860

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2104 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (G) CELL TYPE: human T-cells

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: library 3 day PHA/PEF-BOS

(B) CLONE: human interleukin-12 receptor clone #5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 65..2050

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA 60

TCCG ATG GAG CCG CTG GTG ACC TGG GTG GTC CCC CTC CTC TTC CTC TTC 109

Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe

1 5 10 15

CTG CTG TCC AGG CAG GGC GCT GCC TGC AGA ACC AGT GAG TGC TGT TTT 157

Leu Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe

20 25 30

CAG GAC CCG CCA TAT CCG GAT GCA GAC TCA GGC TCG GCC TCG GGC CCT 205

Gln Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro

35 40 45

AGG GAC CTG AGA TGC TAT CGG ATA TCC AGT GAT CGT TAC GAG TGC TCC 253

Arg Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser

50 55 60

TGG CAG TAT GAG GGT CCC ACA GCT GGG GTC AGC CAC TTC CTG CGG TGT 301

Trp Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys

65 70 75

TGC CTT AGC TCC GGG CGC TGC TGC TAC TTC GCC GCC GGC TCA GCC ACC 349

Cys Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr

	80	85	90	95	
5	AGG CTG CAG TTC TCC GAC CAG GCT GGG GTG TCT GTG CTG TAC ACT GTC				397
	Arg Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val				
	100	105	110		
10	ACA CTC TGG GTG GAA TCC TGG GCC AGG AAC CAG ACA GAG AAG TCT CCT				445
	Thr Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro				
	115	120	125		
15	GAG GTG ACC CTG CAG CTC TAC AAC TCA GTT AAA TAT GAG CCT CCT CTG				493
	Glu Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu				
20	130	135	140		
25	GGA GAC ATC AAG GTG TCC AAG TTG GCC GGG CAG CTG CGT ATG GAG TGG				541
	Gly Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp				
	145	150	155		
30	GAG ACC CCG GAT AAC CAG GTT GGT GCT GAG GTG CAG TTC CGG CAC CGG				589
	Glu Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg				
	160	165	170	175	
35	ACA CCC AGC AGC CCA TGG AAG TTG GGC GAC TGC GGA CCT CAG GAT GAT				637
	Thr Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp				
	180	185	190		
40	GAT ACT GAG TCC TGC CTC TGC CCC CTG GAG ATG AAT GTG GCC CAG GAA				685
	Asp Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu				
45	195	200	205		
50	TTC CAG CTC CGA CGA CGG CAG CTG GGG AGC CAA GGA AGT TCC TGG AGC				733
	Phe Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser				
	210	215	220		

65

	AAG TGG AGC AGC CCC GTG TGC GTT CCC CCT GAA AAC CCC CCA CAG CCT	781
	Lys Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro	
5	225 230 235	
	CAG GTG AGA TTC TCG GTG GAG CAG CTG GGC CAG GAT GGG AGG AGG CGG	829
10	Gln Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg	
	240 245 250 255	
	CTG ACC CTG AAA GAG CAG CCA ACC CAG CTG GAG CTT CCA GAA GGC TGT	877
15	Leu Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys	
	260 265 270	
	CAA GGG CTG GCG CCT GGC ACG GAG GTC ACT TAC CGA CTA CAG CTC CAC	925
20	Gln Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His	
	275 280 285	
25	ATG CTG TCC TGC CCG TGT AAG GCC AAG GCC ACC AGG ACC CTG CAC CTG	973
	Met Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu	
	290 295 300	
30	GGG AAG ATG CCC TAT CTC TCG GGT GCT GCC TAC AAC GTG GCT GTC ATC	1021
	Gly Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile	
35	305 310 315	
	TCC TCG AAC CAA TTT GGT CCT GGC CTG AAC CAG ACG TGG CAC ATT CCT	1069
40	Ser Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro	
	320 325 330 335	
	GCC GAC ACC CAC ACA GAA CCA GTG GCT CTG AAT ATC AGC GTC GGA ACC	1117
45	Ala Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr	
	340 345 350	
50	AAC GGG ACC ACC ATG TAT TGG CCA GCC CGG GCT CAG AGC ATG ACG TAT	1165
	Asn Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr	

55

	355	360	365	
5	TGC ATT GAA TGG CAG CCT GTG GGC CAG GAC GGG GGC CTT GCC ACC TGC			1213
	Cys Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys			
	370	375	380	
10	AGC CTG ACT GCG CCG CAA GAC CCG GAT CCG GCT GGA ATG GCA ACC TAC			1261
	Ser Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr			
	385	390	395	
15	AGC TGG AGT CGA GAG TCT GGG GCA ATG GGG CAG GAA AAG TGT TAC TAC			1309
	Ser Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr			
20	400	405	410	415
25	ATT ACC ATC TTT GCC TCT GCG CAC CCC GAG AAG CTC ACC TTG TGG TCT			1357
	Ile Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser			
	420	425	430	
30	ACG GTC CTG TCC ACC TAC CAC TTT GGG GGC AAT GCC TCA GCA GCT GGG			1405
	Thr Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly			
	435	440	445	
35	ACA CCG CAC CAC GTC TCG GTG AAG AAT CAT AGC TTG GAC TCT GTG TCT			1453
	Thr Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser			
	450	455	460	
40	GTG GAC TGG GCA CCA TCC CTG CTG AGC ACC TGT CCC GGC GTC CTA AAG			1501
	Val Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys			
	465	470	475	
45	GAG TAT GTT GTC CGC TGC CGA GAT GAA GAC AGC AAA CAG GTG TCA GAG			1549
	Glu Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu			
50	480	485	490	495

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	CAT CCC GTG CAG CCC ACA GAG ACC CAA GTT ACC CTC AGT GGC CTG CGG	1597
	His Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg	
5	500 505 510	
	GCT GGT GTA GCC TAC ACG GTG CAG GTG CGA GCA GAC ACA GCG TGG CTG	1645
10	Ala Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu	
	515 520 525	
	AGG GGT GTC TGG AGC CAG CCC CAG CGC TTC AGC ATC GAA GTG CAG GTT	1693
15	Arg Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val	
	530 535 540	
	TCT GAT TGG CTC ATC TTC TTC GCC TCC CTG GGG AGC TTC CTG AGC ATC	1741
20	Ser Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile	
	545 550 555	
	CTT CTC GTG GGC GTC CTT GGC TAC CTT GGC CTG AAC AGG GCC GCA CGG	1789
25	Leu Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg	
	560 565 570 575	
30		
	CAC CTG TGC CCG CCG CTG CCC ACA CCC TGT GCC AGC TCC GCC ATT GAG	1837
35	His Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu	
	580 585 590	
	TTC CCT GGA GGG AAG GAG ACT TGG CAG TGG ATC AAC CCA GTG GAC TTC	1885
40	Phe Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe	
	595 600 605	
	CAG GAA GAG GCA TCC CTG CAG GAG GCC CTG GTG GTA GAG ATG TCC TGG	1933
45	Gln Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp	
	610 615 620	
	GAC AAA GGC GAG AGG ACT GAG CCT CTC GAG AAG ACA GAG CTA CCT GAG	1981
50	Asp Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu	
55		

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5 GGT GCC CCT GAG CTG GCC CTG GAT ACA GAG TTG TCC TTG GAG GAT GGA 2029
Gly Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly
640 645 650 655

10 GAC AGG TGC AAG GCC AAG ATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA 2080
Asp Arg Cys Lys Ala Lys Met
660

15 CTCGCCCCGAG GCTACGTAGC CTTT 2104

20

(2) INFORMATION FOR SEQ ID NO:4:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 662 amino acids
(B) TYPE: amino acid
30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35

(ix) FEATURE:

- (A) NAME/KEY: Region
40 (B) LOCATION: 1..20
(D) OTHER INFORMATION: /note= "N-terminal signal peptide
(1..20 or 23 or 24)"

45

(ix) FEATURE:

- (A) NAME/KEY: Region
50 (B) LOCATION: 541..570
(D) OTHER INFORMATION: /note= "transmembrane region"

55

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 571..662

(D) OTHER INFORMATION: /note= "cytoplasmic tail region"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 52..64

(D) OTHER INFORMATION: /note= "sequence motif of cytokine
receptor superfamily Cys52..Cys62SW"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 222..226

(D) OTHER INFORMATION: /note= "cytokine receptor
superfamily motif (W222SKWS)"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 121..123

(D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 329..331

(D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 346..348

(D) OTHER INFORMATION: /note= "N-linked glycosylation"

site"

5 (ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 352..354

10 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

(ix) FEATURE:

15 (A) NAME/KEY: Region

(B) LOCATION: 442..444

20 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

(ix) FEATURE:

25 (A) NAME/KEY: Region

(B) LOCATION: 456..458

30 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

(ix) FEATURE:

35 (A) NAME/KEY: Region

(B) LOCATION: 24..540

(D) OTHER INFORMATION: /note= "Extracellular region"

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

45 Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe Leu
1 5 10 15

50 Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe Gln
20 25 30

55

Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg

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Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp

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Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys

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80

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg

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Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr

100

105

110

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu

115

120

125

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly

130

135

140

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu

145

150

155

160

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr

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170

175

Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp

180

185

190

Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe

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Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys

	210	215	220	
5	Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln			
	225	230	235	240
10	Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu			
	245	250	255	
15	Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln			
	260	265	270	
20	Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His Met			
	275	280	285	
25	Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly			
	290	295	300	
30	Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser			
	305	310	315	320
35	Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala			
	325	330	335	
40	Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn			
	340	345	350	
45	Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys			
	355	360	365	
50	Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser			
	370	375	380	
55	Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser			
	385	390	395	400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile

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Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr

420

425

430

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Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr

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Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val

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Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu

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Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu His

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495

30

Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala

500

505

510

35

Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg

515

520

525

40

Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser

530

535

540

45

Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu

545

550

555

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Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His

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Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe
580 585 590

Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln
595 600 605

Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp
610 615 620

Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly
625 630 635 640

Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp
645 650 655

Arg Cys Lys Ala Lys Met
660

Claims

1. A low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which

- (a) has low binding affinity for IL-12, and
- (b) when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12.

2. The protein of claim 1, wherein the IL-12 beta2 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1.

3. The protein of claim 2 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:2.

4. The protein of claim 3, wherein the IL-12 beta2 receptor protein has SEQ ID NO:2 or allelic forms or variants thereof.

5. The protein of any one of claims 1 to 4 encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined in any one of the preceding claims, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of Ig other than the first domain of said constant region.

6. A complex capable of binding to IL-12 with high affinity, comprising interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof as defined in any of claims 1 - 4 complexed with IL-12 beta1 receptor protein, or a fragment thereof which

- (a) has low binding affinity for IL-12, and
- (b) when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

7. The complex of claim 6, wherein the IL-12 beta1 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3.
8. The protein of claim 7 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:4.
9. The protein of claim 8, wherein the IL-12 beta1 receptor protein has SEQ ID NO:4 or allelic forms or variants thereof.
10. A protein encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of any one of claims 1 to 4 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a protein of any of claims 7 to 9 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.
11. A protein or complex of any one of claims 1 to 10 which is soluble.
12. Nucleic acids which encode a protein or complex of any one of claims 1 - 11.
13. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta2 receptor protein having the SEQ ID NO:1.
14. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta1 receptor protein having the SEQ ID NO:3.
15. A vector comprising a nucleic acid of any one of claims 12 to 14.
16. An expression vector comprising a nucleic acid of any one of claims 12 - 14 operably linked to control sequences recognised by a host cell.
17. A host cell transformed with a nucleic acid of any one of claims 12 to 16.
18. The host cell of claim 17 wherein the protein or complex is expressed on its surface.
19. The host cell of claim 18 wherein the host cell proliferates in the presence of IL-12.
20. The host cell of claims 17 - 19, wherein the host cell is transformed with a first vector comprising a nucleic acid encoding the protein as defined in claim 1 and a second vector comprising a nucleic acid encoding the protein as defined in claim 7 or with a single vector comprising a nucleic acid encoding the protein as defined in claim 1 and a nucleic acid encoding a protein as defined in claim 7.
21. An antibody directed against a protein of any of claims 1 to 11.
22. A process for the preparation of a protein of any of claims 1 to 11 which comprises the expression of a nucleic acid of any one of claims 12 to 14 in a suitable host cell.
23. A pharmaceutical composition comprising a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 and a pharmaceutically acceptable carrier.
24. The pharmaceutical composition of claim 23 which further comprises a therapeutically effective amount of one or more cytokine antagonists.
25. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament.
26. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament for the treatment of autoimmune dysfunction.

27. A method for screening compounds useful for inhibition of IL-12 activity, comprising

- 5 a) contacting a compound suspected of inhibiting IL-12 activity to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
 b) detection of the inhibiting effect.

28. A method for screening compounds useful as agonists of IL-12, comprising

- 10 a) contacting a compound suspected of being an IL-12 agonist to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
 b) detection of an agonist effect.

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Fig. 1

10	20	30	40	50	60
TGCAGAGAAC	AGAGAAAGGA	CATCTGCGAG	GAAAGTTCCC	TGATGGCTGT	CAACAAAGTG
70	80	90	100	110	120
CCACGTCTCT	ATGGCTGTGT	ACGCTGAGCA	CACGATTTTA	TCGCGCCTAT	CATATCTTGG
130	140	150	160	170	180
TGCATAAACG	CACCTCACCT	CGGTCAACCC	TTGCTCCGTC	TTATGAGACA	GGCTTTATTA
190	200	210	220	230	240
TCCGCATTTT	ATATGAGGGG	AATCTGACGG	TGGAGAGAGA	ATTATCTTGC	TCAAGGCGAC
250	260	270	280	290	300
ACAGCAGAGC	CCACAGGTGG	CAGAATCCCA	CCCGAGCCCG	CTTCGACCCG	CGGGGTGGAA
310	320	330	340	350	360
ACCACGGGCG	CCCGCCCGGC	TGCGCTTCCA	GAGCTGAACT	GAGAAGCGAG	TCCTCTCCGC
370	380	390	400	410	420
CCTGCGGCCA	CCGCCAGCC	CCGACCCCG	CCCCGGCCCG	ATCCTCACTC	GCCGCCAGCT
430	440	450	460	470	480
CCCCGCGCCC	ACCCCGGAGT	TGGTGGCGCA	GAGGCGGGAG	GCGGAGGCGG	GAGGGCGGGC
490	500	510	520	530	540
GCTGGCACCG	GGAACGCCCC	AGCGCCGGCA	GAGAGCGCGG	AGAGCGCGAC	ACGTGCGGCC
550	560	570	580	590	600
CAGAGCACCG	GGGCCACCCG	GTCCCCGAG	GCCCCGGACC	GCGCCCGCTG	GCAGGCGACA
610	620	630	640	650	660
CGTGGAAGAA	TACGGAGTTC	TATACCAGAG	TTGATTGTG	<u>ATGGCACATA</u>	CTTTTAGAGG
670	680	690	700	710	720
ATGCTCATTG	GCATTTATGT	TTATAATCAC	GTGGCTGTG	ATTAAAGCAA	AAATAGATGC
730	740	750	760	770	780
GTGCAAGAGA	GGCGATGTGA	CTGTGAAGCC	TTCCCATGTA	ATTTTACTTG	GATCCACTGT
790	800	810	820	830	840
CAATATTACA	TGCTCTTTGA	AGCCCAGACA	AGGCTGCTTT	CACTATTCCA	GACGTAACAA
850	860	870	880	890	900
GTTAATCCTG	TACAAGTTTG	ACAGAAGAAT	CAATTTTCAC	CATGGCCACT	CCCTCAATTC
910	920	930	940	950	960
TCAAGTCACA	GGTCTTCCCC	TTGGTACAAC	CTTGTTTGTC	TGCAAAGTGG	CCTGTATCAA
970	980	990	1000	1010	1020
TAGTGATGAA	ATTCAAATAT	GTGGAGCAGA	GATCTTCGTT	GGTGTTGCTC	CAGAACAGCC

Fig. 1 CONT'D

1030	1040	1050	1060	1070	1080
TCAAAATTTA	TCCTGCATAC	AGAAGGGAGA	ACAGGGGACT	GTGGCCTGCA	CCTGGGAAAAG
1090	1100	1110	1120	1130	1140
AGGACGAGAC	ACCCACTTAT	ACACTGAGTA	TACTCTACAG	CTAAGTGGAC	CAAAAAATTT
1150	1160	1170	1180	1190	1200
AACCTGGCAG	AAGCAATGTA	AAGACATTTA	TTGTGACTAT	TTGGACTTTG	GAATCAACCT
1210	1220	1230	1240	1250	1260
CACCCCTGAA	TCACCTGAAT	CCAATTTTAC	AGCCAAGGTT	ACTGCTGTCA	ATAGTCTTGG
1270	1280	1290	1300	1310	1320
AAGCTCCTCT	TCACCTCCAT	CCACATTCAC	ATTCTTGGAC	ATAGTGAGGC	CTCTTCCTCC
1330	1340	1350	1360	1370	1380
GTGGGACATT	AGAATCAAAT	TTCAAAAGGC	TTCCGTGAGC	AGATGTACCC	TTTATTGGAG
1390	1400	1410	1420	1430	1440
AGATGAGGGA	CTGGTACTGC	TTAATCGACT	CAGATATCGG	CCCAGTAACA	GCAGGCTCTG
1450	1460	1470	1480	1490	1500
GAATATGGTT	AATGTTACAA	AGGCCAAAAG	AAGACATGAT	TTGCTGGATC	TGAAACCATT
1510	1520	1530	1540	1550	1560
TACAGAATAT	GAATTTCAGA	TTTCCTCTAA	GCTACATCTT	TATAAGGGAA	GTTGGAGTGA
1570	1580	1590	1600	1610	1620
TTGGAGTGAA	TCATTGAGAG	CACAAACACC	AGAAGAAGAG	CCTACTGGGA	TGTTAGATGT
1630	1640	1650	1660	1670	1680
CTGGTACATG	AAACGGCACA	TTGACTACAG	TAGACAACAG	ATTTCTCTTT	TCTGGAAGAA
1690	1700	1710	1720	1730	1740
TCTGAGTGTC	TCAGAGGCAA	GAGGAAAAAT	TCTCCACTAT	CAGGTGACCT	TGCAGGAGCT
1750	1760	1770	1780	1790	1800
GACAGGAGGG	AAAGCCATGA	CACAGAACAT	CACAGGACAC	ACCTCCTGGA	CCACAGTCAT
1810	1820	1830	1840	1850	1860
TCCTAGAACC	GGAAATTGGG	CTGTGGCTGT	GTCTGCAGCA	AATTCAAAAG	GCAGTTCTCT
1870	1880	1890	1900	1910	1920
GCCCACTCGT	ATTAACATAA	TGAACCTGTG	TGAGGCAGGG	TTGCTGGCTC	CTCGCCAGGT
1930	1940	1950	1960	1970	1980
CTCTGCAAAC	TCAGAGGGCA	TGGACAACAT	TCTGGTGAAT	TGGCAGCCTC	CCAGGAAAGA
1990	2000	2010	2020	2030	2040
TCCCTCTGCT	GTTCAGGAGT	ACGTGGTGGA	ATGGAGAGAG	CTCCATCCAG	GGGGTGACAC

Fig. 1 CONT'D

2050	2060	2070	2080	2090	2100
ACAGGTCCCT	CTAAACTGGC	TACGGAGTCG	ACCCTACAAT	GTGTCTGCTC	TGATTTCAGA
2110	2120	2130	2140	2150	2160
GAACATAAAA	TCCTACATCT	GTTATGAAAT	CCGTGTGTAT	GCACTCTCAG	GGGATCAAGG
2170	2180	2190	2200	2210	2220
AGGATGCAGC	TCCATCCTGG	GTAACCTCTAA	GCACAAAGCA	CCACTGAGTG	GCCCCCACAT
2230	2240	2250	2260	2270	2280
TAATGCCATC	ACAGAGGAAA	AGGGGAGCAT	TTTAATTTCA	TGGAACAGCA	TTCCAGTCCA
2290	2300	2310	2320	2330	2340
GGAGCAAATG	GGCTGCCTCC	TCCATTATAG	GATATACTGG	AAGGAACGGG	ACTCCAACCTC
2350	2360	2370	2380	2390	2400
CCAGCCTCAG	CTCTGTGAAA	TTCCCTACAG	AGTCTCCCAA	AATTCACATC	CAATAAACAG
2410	2420	2430	2440	2450	2460
CCTGCAGCCC	CGAGTGACAT	ATGTCCTGTG	GATGACAGCT	CTGACAGCTG	CTGGTGAAAG
2470	2480	2490	2500	2510	2520
TTCCACCGGA	AATGAGAGGG	AATTTTGTCT	GCAAGGTAAA	GCCAATTGGA	TGGCGTTTGT
2530	2540	2550	2560	2570	2580
GGCACCAAGC	ATTTGCATTG	CTATCATCAT	GGTGGGCATT	TTCTCAACGC	ATTACTTCCA
2590	2600	2610	2620	2630	2640
GCAAAAGGTG	TTGTCTCTCC	TAGCAGCCCT	CAGACCTCAG	TGGTGTAGCA	GAGAAATTCC
2650	2660	2670	2680	2690	2700
AGATCCAGCA	AATAGCACTT	GCGCTAAGAA	ATATCCCATT	GCAGAGGAGA	AGACACAGCT
2710	2720	2730	2740	2750	2760
GCCCTTGGAC	AGGCTCCTGA	TAGACTGGCC	CACGCCTGAA	GATCCTGAAC	CGCTGGTCAT
2770	2780	2790	2800	2810	2820
CAGTGAAGTC	CTTCATCAAG	TGACCCAGT	TTTCAGACAT	CCCCCTGCT	CCAACCTGGCC
2830	2840	2850	2860	2870	2880
ACAAAGGGAA	AAAGGAATCC	AAGGTCATCA	GGCCTCTGAG	AAAGACATGA	TGCACAGTGC
2890	2900	2910	2920	2930	2940
CTCAAGCCCA	CCACCTCCAA	GAGCTCTCCA	AGCTGAGAGC	AGACAACTGG	TGGATCTGTA
2950	2960	2970	2980	2990	3000
CAAGGTGCTG	GAGAGCAGGG	GCTCCGACCC	AAAGCCAGAA	AACCCAGCCT	GTCCCTGGAC
3010	3020	3030	3040	3050	3060
GGTGCTCCCA	GCAGGTGACC	TTCCCACCCA	TGATGGCTAC	TTACCCTCCA	ACATAGATGA

Fig. 1 CONT'D

3070	3080	3090	3100	3110	3120
CCTCCCCTCA	CATGAGGCAC	CTCTCGCTGA	CTCTCTGGAA	GAAGTGGAGC	CTCAGCACAT
3130	3140	3150	3160	3170	3180
CTCCCTTTCT	GTTTTCCCCT	CAAGTTCTCT	TCACCCACTC	ACCTTCTCCT	GTGGTGATAA
3190	3200	3210	3220	3230	3240
GCTGACTCTG	GATCAGTTAA	AGATGAGGTG	TGACTCCCTC	ATGCTCTGAG	TGGTGAGGCT
3250	3260	3270	3280	3290	3300
TCAAGCCTTA	AAGTCAGTGT	GCCCTCAACC	AGCACAGCCT	GCCCCAATTC	CCCCAGCCCC
3310	3320	3330	3340	3350	3360
TGCTCCAGCA	GCTGTCATCT	CTGGGTGCCA	CCATCGGTCT	GGCTGCAGCT	AGAGGACAGG
3370	3380	3390	3400	3410	3420
CAAGCCAGCT	CTGGGGGAGT	CTTAGGAACT	GGGAGTTGGT	CTTCACTCAG	ATGCCTCATC
3430	3440	3450	3460	3470	3480
TTGCCTTTCC	CAGGGCCTTA	AAATTACATC	CTTCACTGTG	TGGACCTAGA	GACTCCAAC
3490	3500	3510	3520	3530	3540
TGAATTCCTA	GTAACTTTCT	TGGTATGCTG	GCCAGAAAGG	GAAATGAGGA	GGAGAGTAGA
3550	3560	3570	3580	3590	3600
AACCACAGCT	CTTAGTAGTA	ATGGCATACA	GTCTAGAGGA	CCATTCATGC	AATGACTATT
3610	3620	3630	3640	3650	3660
TCTAAAGCAC	CTGCTACACA	GCAGGCTGTA	CACAGCAGAT	CAGTACTGTT	CAACAGAACT
3670	3680	3690	3700	3710	3720
TCCTGAGATG	ATGGAAATGT	TCTACCTCTG	CACTCACTGT	CCAGTACATT	AGACACTAGG
3730	3740	3750	3760	3770	3780
CACATTGGCT	GTTAATCACT	TGGAATGTGT	TTAGCTTGAC	TGAGGAATTA	AATTTTGATT
3790	3800	3810	3820	3830	3840
GTAATTTTAA	ATCGCCACAC	ATGGCTAGTG	GCTACTGTAT	TGGAGTGCAC	AGCTCTAGAT
3850	3860	3870	3880	3890	3900
GGCTCCTAGA	TTATTGAGAG	CCTCCAAAAC	AAATCAACCT	AGTTCTATAG	ATGAAGACAT
3910	3920	3930	3940	3950	3960
AAAAGACACT	GGTAAACACC	AATGTAAAAG	GGCCCCCAAG	GTGGTCATGA	CTGGTCTCAT
3970	3980	3990	4000	4010	4020
TTGCAGAAGT	CTAAGAATGT	ACCTTTTTCT	GGCCGGGCGT	GGTAGCTCAT	GCCTGTAATC
4030	4040				
CCAGCACTTT	GGGAGGCTGA				

Fig. 2

1 MAHTFRGCSL AFMFIITWLL IKAKIDACKR GDVTVKPSHV ILLGSTVNIT
 51 CSLKPRQGGCF HYSRRNKLIL YKFDRRINFH HGHSLSNSQVT GLPLGTTLFV
 101 CKLACINSDE IQICGAEIFV GVAPEQPQNL SCIQKGEQGT VACTWERGRD
 151 THLYTEYTLQ LSGPKNLTWQ KQCKDIYCDY LDFGINLTPE SPESNFTAKV
 201 TAVNSLGSSS SLPSTFTFLD IVRPLPPWDI RIKFQKASVS RCTLYWRDEG
 251 LVLLNRLRYR PSNSRLWNMV NVTKAKGRHD LLDLKPFTEY EFQISSKLHL
 301 YKGSWSDWSE SLRAQTPEEE PTGMLDVWYM KRHIDYSRQQ ISLFWKNLSV
 351 SEARGKILHY QVTQLQELTGG KAMTQNLTGH TSWTTVIPRT GNWAVAVSAA
 401 NSKGSSLPTR INIMNLCEAG LLAPRQVSAN SEGMDNILVT WQPPRKDPSA
 451 VQEYVVEWRE LHPGGDTQVP LNWLSRPYN VSALISENIK SYICYEIRVY
 501 ALSGDQGGCS SILGNSKHKA PLSGPHINAI TEEKGSILIS WNSIPVQEQM
 551 GCLLHYRIYW KERDSNSQPQ LCEIPYRVSQ NSHPINSIQP RVTYVLWMTA
 601 LTAAGESSHG NEREFCLQCK ANWMAFVAPS ICIAIMVGI FSTHYFQQKV
 651 FVLLAALRPQ WCSREIPDFA NSTCAKKYPI AEKTQLPLD RLLIDWPTPE
 701 DPEELWISEV LHQVTPVFRH PPCSNWPQRE KGIQGHQASE KDMMSASSP
 751 PPPRALQAES RQLVDLYKVL ESRGSDPKPE NPACPWTVLP AGDLPTHDGY
 801 LPSNIDDLPS HEAPLADSLE ELEPQHISLS VFPSSSLHPL TFSCGDKLTL
 851 DQLKMRCDL ML

Fig. 3

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      10      20      30      40      50      60      70
GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCCTGGG GCTGTGGGGC TCTACGTGGA TCCGATGGAG

      80      90     100     110     120     130     140
CCGCTGGTGA CCTGGGTGGT CCCCCTCCTC TTCCTCTTCC TGCTGTCCAG GCAGGGCGCT GCCTGCAGAA

     150     160     170     180     190     200     210
CCAGTGAGTG CTGTTTTCAG GACCCGCCAT ATCCGGATGC AGACTCAGGC TCGGCCTCGG GCCCTAGGGA

     220     230     240     250     260     270     280
CCTGAGATGC TATCGGATAT CCAGTGATCG TTACGAGTGC TCCTGGCAGT ATGAGGGTCC CACAGCTGGG

     290     300     310     320     330     340     350
GTCAGCCACT TCCTGCCGTG TTGCCTTAGC TCCGGGCGCT GCTGCTACTT CGCCGCCGGC TCAGCCACCA

     360     370     380     390     400     410     420
GGCTGCAGTT TCCCGACCAG GCTGGGGTGT CTGTGCTGTA CACTGTCACA CTCTGGGTGG AATCCTGGGC

     430     440     450     460     470     480     490
CAGGAACCAG ACAGAGAAGT CTCCTGAGGT GACCCCTGCAG CTCTACAACT CAGTTAAATA TGAGCCTCCT

     500     510     520     530     540     550     560
CTGGGAGACA TCAAGGTGTC CAAGTTGGCC GGGCAGCTGC GTATGGAGTG GGAGACCCCG GATAACCAGG

     570     580     590     600     610     620     630
TTGGTGCTGA GGTGCAGTTC CGGCACCGGA CACCCAGCAG CCCATGGAAG TTGGGCGACT GCGGACCTCA

     640     650     660     670     680     690     700
GGATGATGAT ACTGAGTCCT GCCTCTGCCC CCTGGAGATG AATGTGGCCC AGGAATTCCA GCTCCGACGA

     710     720     730     740     750     760     770
CGGCAGCTGG CGAGCCAAGG AAGTTCCTGG AGCAAGTGA GCAGCCCCGT GTCCCTTCCC CCTGAAAACC

     780     790     800     810     820     830     840
CCCCACAGCC TCAGGTGAGA TTCTCGGTGG AGCAGCTGGG CCAGGATGGG AGGAGGCGGC TGACCCTGAA

     850     860     870     880     890     900     910
AGAGCAGCCA ACCCAGCTGG AGCTTCCAGA AGGCTGTCAA GGGCTGGCGC CTGGCACGGA GGTCACCTAC

     920     930     940     950     960     970     980
CGACTACAGC TCCACATGCT GTCTGCCCCG TGTAAGGCCA AGGCCACCAG GACCCCTGCAC CTGGGGAAGA

     990    1000    1010    1020    1030    1040    1050
TGCCCTATCT CTGGGTGCT GCCTACAACG TGGCTGTGAT CTCCTCGAAC CAATTGGTTC CTGGCCTGAA

    1060    1070    1080    1090    1100    1110    1120
CCAGACGTGG CACATTCTCG CCGACACCCA CACAGAACCA GTGGCTCTGA ATATCAGCGT CGGAACCAAC

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Fig. 3 CONT'D

1130	1140	1150	1160	1170	1180	1190
GGGACCACCA	TGTATTGGCC	AGCCCCGGGCT	CAGAGCATGA	CGTATTGCAT	TGAATGGCAG	CCTGTGGGCC
1200	1210	1220	1230	1240	1250	1260
AGGACGGGGG	CCTTGCCACC	TGCAGCCTGA	CTGCGCCGCA	AGACCCGGAT	CCGGCTGGAA	TGGCAACCTA
1270	1280	1290	1300	1310	1320	1330
CAGCTGGAGT	CGAGAGTCTG	GGGCAATGGG	GCAGGAAAAG	TGTTACTACA	TTACCATCTT	TGCCTCTCGG
1340	1350	1360	1370	1380	1390	1400
CACCCCGAGA	AGCTCACCTT	GTGGTCTACG	GTCTGTGTTA	CCTACCACTT	TGGGGGCAAT	GCCTCAGCAG
1410	1420	1430	1440	1450	1460	1470
CTGGGACACC	GCACCAAGTC	TCGGTGAAGA	ATCATAGCTT	GGACTCTGTG	TCTGTGGACT	GGGCACCATC
1480	1490	1500	1510	1520	1530	1540
CCTGCTGAGC	ACCTGTCCCG	GCGTCCTAAA	GGAGTATGTT	GTCCGCTGCC	GAGATGAAGA	CAGCAAAACA
1550	1560	1570	1580	1590	1600	1610
GTGTCAGAGC	ATCCCGTGCA	GCCCCAGAG	ACCCAAGTTA	CCCTCAGTGG	CCTGCGGGCT	GGTGTAGCCT
1620	1630	1640	1650	1660	1670	1680
ACACGGTGCA	GGTGCGAGCA	GACACAGCGT	GGCTGAGGGG	TGTCTGGAGC	CAGCCCCAGC	GCTTCAGCAT
1690	1700	1710	1720	1730	1740	1750
CGAAGTGAG	GTTTCTGATT	GGCTCATCTT	CTTCGCTTCC	CTGGGGAGCT	TCCTGAGCAT	CCTTCTCGTG
1760	1770	1780	1790	1800	1810	1820
GGCGTCCTTG	GCTACCTTGG	CCTGAACAGG	GCCGCACGGC	ACCTGTGCCC	GCCGCTGCCC	ACACCCTGTG
1830	1840	1850	1860	1870	1880	1890
CCAGTCCCGC	CATTGAGTTC	CCTGGAGGGA	AGGAGACTTG	GCAGTGGATC	AACCCAGTGG	ACTTCCAGGA
1900	1910	1920	1930	1940	1950	1960
AGAGGCATCC	CTGCAGGAGG	CCCTGGTGGT	AGAGATGTCC	TGGGACAAAG	GCGAGAGGAC	TGAGCCTCTC
1970	1980	1990	2000	2010	2020	2030
GAGAAGACAG	AGCTACCTGA	GGGTGCCCCCT	GAGCTGGCCC	TGGATACAGA	GTTGTCCCTG	GAGGATGGAG
2040	2050	2060	2070	2080	2090	2100
ACAGGTGCAA	GGCCAAGATG	<u>TGATCGTTGA</u>	GGCTCAGAGA	GGGTGAGTGA	CTCGCCCGAG	GCTACGTAGC

Fig. 4

10	20	30	40	50	60	70
MEPLVTWVVP	LELELLSRQ	GAACRTSECC	FQPPYPDAD	SGSASGRDL	RCYRISSDRY	ECSWQYEGPT
80	90	100	110	120	130	140
AGVSHFLRCC	LSSGRCCYFA	AGSATRLQFS	DQAGVSVLYT	VTLWVESWAR	NQTEKSPEVT	LQLYNSVKYE
150	160	170	180	190	200	210
PPLGDIKVS	KLAGQLRMEWE	TPDNQVGAEV	QFRHRTSPSP	WKLGDGCPQD	DDTESCLCPL	EMNVAQEFQL
220	230	240	250	260	270	280
RRRQLGSQGS	SWSKWSSPVC	VPPENPPQPQ	VRFSVEQLGQ	DGRRRLTLKE	QPTQLELPEG	CQGLAPGTEV
290	300	310	320	330	340	350
TYRLQLHMLS	CPCKAKATRT	LHLGKMPYLS	GAAYNVAVIS	SNQFGPGLNQ	TWHIPADTHT	EPVALNISVG
360	370	380	390	400	410	420
TNGTTMYWPA	RAQSMTYCIE	WQPVGQDGG	ATCSLTAPQD	PDPAGMATYS	WSRESGAMGQ	EKCYVITIFA
430	440	450	460	470	480	4
SAHPEKLTW	STVLSTYHFG	GNASAAGTPH	HVSVKNHSLD	SVSVDWAPSL	LSTCPGVLKE	YVVRCRDEDS
500	510	520	530	540	550	560
KQVSEHPVQP	TETQVTLISGL	RAGVAYTVQV	RADTAWLRGV	WSQPQRFSE	VQVSDWLIFF	ASLGSFLSIL
570	580	590	600	610	620	630
LVGVLYLGL	NRAARHLCP	LPTPCASSAI	EFPGGKETWQ	WINPVPDEEE	ASLQEAALYVE	MSWDKGERTE
640	650	660				
PLEKTELPEG	APELALDTEL	SLEDGDRCKA	KM			

Fig. 5

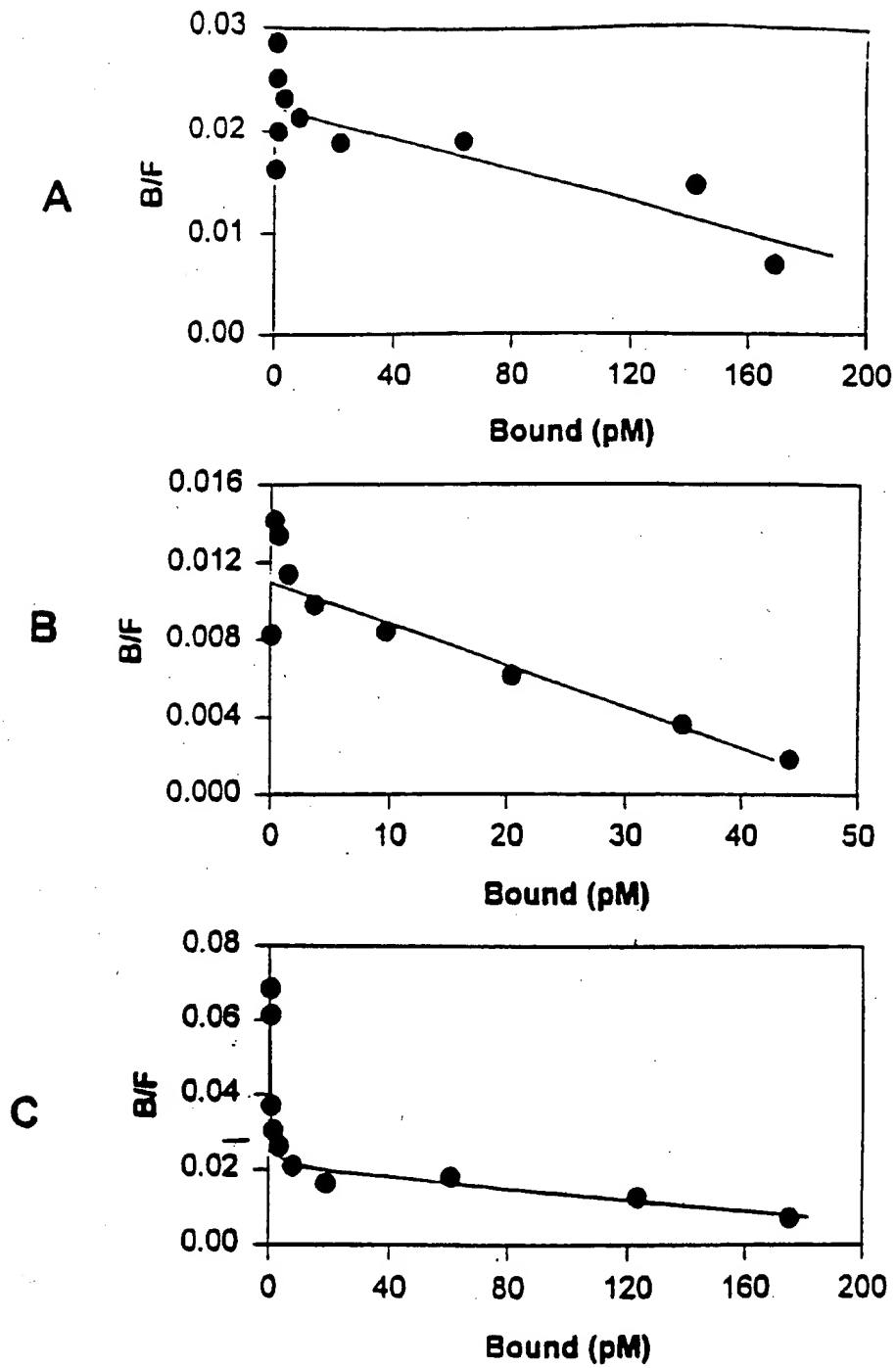


Fig. 6

